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ANNOTATED LIST OF SPECIES

First records of protosteloid amoebae isolated from coastal litter in the Philippines

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Abstract. The distribution and ecology of protosteloid amoebae are still relatively unexplored, especially in the paleotropics, despite their role in understanding the evolutionary lineage of Eumycetozoans, more commonly known as slime molds. Hence, an occurrence survey was carried out for the first time in the coastal vegetation of San Fernando City, La Union, Philippines to generate a species listing of protosteloid amoebae. Herein, a total of 40 substrate samples, comprising 20 aerial litter (AL) and 20 ground litter (GL), were collected from four collection points along the coastline of the study site to prepare for their inoculation in weak malt yeast agar (wMYA) plates. The culture plates recovered 12 species belonging to nine genera, all of which are reported as new records of protosteloid amoebae for the Philippines. This is the first study to be ever conducted on the occurrence of protosteloid amoebae in Southeast Asia.

Key words. Fruiting bodies, litter ecology, protist, species checklist, sporocarps

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INTRODUCTION

Protosteloid amoebae represent a paraphyletic group of diverse slime molds with amoeboid trophic cells and simple fruiting bodies in their life cycles. Together with myxomycetes and dictyostelids, this category of slime molds forms the taxonomic group, Eumycetozoa. However, despite their role in understanding the evolutionary lineage of Eumycetozoans, protosteloid amoebae have received considerably less attention compared to the other two groups (Olive 1975; Adl et al. 2005; Spiegel et al. 2007). Protosteloid amoebae have simple fructifications that range from 5 μm for the smallest species to more than 250 μm for some of the largest examples (Shadwick et al. 2009b). They consist of single or multiple spores of 5–40 μm in diameter and are accompanied by a single delicate acellular stalk that completes a typical structure of protosteloid amoebae (Tesmer et al. 2005). As microbivorous predators that prey on bacteria, yeast, and spores of filamentous fungi, protosteloid amoebae are conveyed to play a role in maintaining ecological balance in microbial communities in various habitats (Aguilar et al. 2011; Gül and Ergül 2017).

To date, there are 37 previously described and some undescribed protosteloid amoebae known to exist (Spiegel 1989; Spiegel et al. 1995a, 2004). Most surveys investigating occurrences of this group of species were carried out in various parts of North America and the Neotropics, with records having been reported in the beech and pine forests of Ohio (Best and Spiegel 1984), tropical ecosystems of Puerto Rico (Stephenson et al. 1999; Moore and Spiegel 2000b) and Costa Rica (Moore and Stephenson 2003), in boreal forests and tundra of Alaska (Moore et al. 2000), and in the temperate zones of Arkansas (Moore and Spiegel 2000a). Protosteloid amoebae were also known to have been isolated from aquatic environments in the states of Arkansas and Oklahoma (Lindley et al. 2007). Beyond these regions, there are reports of protosteloid amoebae outside North America and the Neotropics. For example, protosteloid amoebae were isolated from various substrates (including litter and bark) collected from the Russian Federation (Kosheleva et al. 2009), Germany (Tesmer et al. 2005; Tesmer and Schnittler 2009), and Turkey (Gül and Ergül 2017).

Contrariwise, investigations on the distribution and ecology of protosteloid amoebae assemblages in the paleotropics are still relatively unexplored, with only one study to have ever been carried out in northern India (Shadwick and Stephenson 2004). Since then, studies on protosteloid amoebae have neither been

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published in Asia nor reported in Southeast Asia. This warrants an occurrence investigation of protosteloid amoebae in this region to serve as baseline research for further ecological studies under the Paleotropical context. As such, the objective of this study was to establish the first species listing of protosteloid amoebae isolated in aerial and ground-litter substrates collected along the coastline of San Fernando, La Union, Philippines.

STUDY AREA

This study was carried out based on four collection points designated along the coastline of San Fernando City, La Union, Philippines, during the wet season in July 2023. The chosen study site is characterized by sparsely occupied mangrove and coconut trees near MacArthur Highway (Figure 1).

METHODS

At each collection point, litter samples were collected from aerial and ground microhabitats designating the samples as either aerial litter or ground litter, respectively. On one hand, aerial-litter samples were defined as dead litter that were collected above ground or those primarily still attached to plant parts. On the other hand, ground-litter samples were collected based on dead litter lying on the ground. Collected samples were placed inside paper envelopes, air-dried, and transported to the University of Santo Tomas, Philippines – Central Laboratory.

Primary Isolation plates (PIP) were prepared following a modification of the technique previously described by Olive (1975). The air-dried aerial and ground-litter samples were cut into small strips (ca. 2–2.5 × 1 cm) and soaked completely in sterile, distilled water for 20 minutes. Eight pieces from each sample were plated about halfway from the center to the edges of the plates, forming a circle on a 9 cm petri plate (Figure 2) containing weak malt yeast agar medium (0.02 g malt extract, 0.02 g yeast extract, 0.75 g K_2HPO, 15 g agar/L of distilled water). The cultures were incubated at room temperature for at least three days and were examined with a compound microscope using a 10× objective by scanning the edge of each piece of the cut litter substrate for fruiting bodies of protosteloid amoebae. Observations were done over a span of 2 weeks from the start of the incubation period.

Characterization and species identification of each protosteloid amoeba was based on the morphology of the fruiting body upon observation using Cambridge Instruments Galen III brightfield microscope at 100× total magnification, focusing primarily on the size and shape of spores, spore multiplicity, as well as length and thickness of the stalk of the sporocarp. Additional morphological descriptors that may help establish the identity of the species include the deciduousness of the sporocarps as well as their response to air currents which was done by introducing air into the PIP using a bulb aspirator. Morphological comparisons were performed using a compendium by Spiegel et al. (2007), as well as other published articles (Aguilar

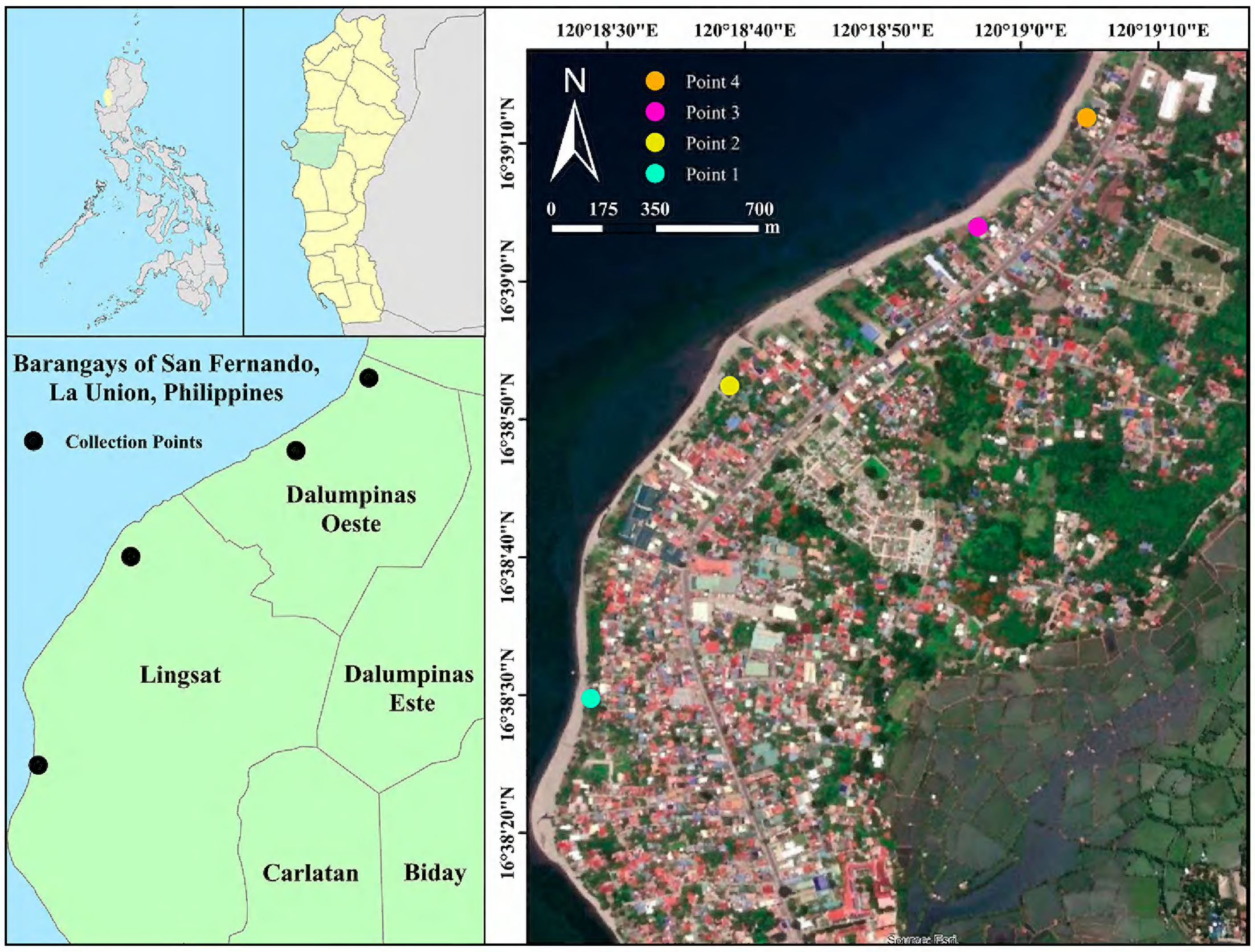

Figure 1. Location of collection points within the San Fernando, La Union, Philippines.

Figure 2. Agar plate setup inclusive of small strips of leaf litter for the isolation and cultivation of protosteloid amoebae.

and Spiegel 2007; Ndiritu et al. 2009), as a reference. Currently accepted names were checked and are based on the online nomenclatural database available for eumycetozoans (https://eumycetozoa.com/data/index.php).

For each collection point, five aerial litter and five ground litter were collected, rendering a total of 40 substrate samples (20 ground-litter samples and 20 aerial-litter samples). Occurrence records were based on the presence of a species on a single strip counted as one record, regardless of the number of sporocarps or colonies observed in that strip throughout the observation period. The exhaustiveness of the survey was assessed by using richness estimators encompassing abundance-based coverage (ACE) and incidence-based coverage (ICE). These estimators were preferred for this setup such that they can account for abundant species that are expected to occur in any given sample, which is true for certain protosteloid amoebae. ACE relies on the number of individuals for each species, while ICE, on the other hand, utilizes the number of species for each sample (Ndiritu et al. 2009). The estimators for each collection point and microhabitat were acquired by using the *wiqid* R package (Juat et al. 2022) in RStudio. The sampling effort of the survey was quantified by dividing the number of recorded species by the highest number of expected species as estimated by either ACE or ICE multiplied by 100%.

RESULTS

A total of 12 species of protosteloid amoebae belonging to nine genera were identified from litter-based PIPs recovered from the survey (Table 1). All 12 species are newly recorded from the Philippines and Southeast Asia. Of the 40 PIPs, 15 (38%) were positive for the presence of protosteloid amoebae. *Protostelium mycophagum* Olive & Stoian. (Figure 3G) and *Cavostelium apophysatum* Olive (Figure 3B) were established to be the most widely distributed species in the study site having been reported in all collection points in both aerial and ground litter.

Genus *Acanthamoeba* Volkonsky 1931

Acanthamoeba pyriformis (Olive & Stoian.) Spiegel & Shadwick 2016

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 2; 16.6479°N, 120.3108°E; N.H.A. Dagamac leg.; ground litter, 1 record, S2G4.

Identification. This deciduous species is denoted by its variable sporocarp size similar to that of *P. mycophagum*. Its obpyriform or campanulate spore is a result of a socket located in its base which connects to a long, gently tapered stalk.

Table 1. Occurrences of protosteloid amoebae species for each collection point and microhabitat.

	Point 1	Point 2	Point 3	Point 4	Aerial litter	Ground litter	TI*	NCP*
Acanthamoeba pyriformis	—	1	—	—	—	1	1	1
Cavostelium apophysatum	1	1	5	3	8	2	10	4
Echinostelium bisporum	—	—	1	—	1	—	1	1
Luapeleamoeba arachispora	—	—	1	—	1	—	1	1
Microglomus paxillus	—	1	2	—	3	—	3	1
Nematostelium ovatum	2	—	1	—	—	3	3	2
Protostelium mycophagum	1	2	6	1	5	5	10	4
Protostelium nocturnum	—	—	2	4	—	6	6	2
Schizoplasmodiopsis amoeboidea	—	—	2	—	2	—	2	1
Schizoplasmodiopsis pseudoendospora	—	6	—	—	—	6	6	1
Schizoplasmodiopsis vulgaris	—	1	2	—	1	2	3	2
Tychosporium acutostipes	—	—	1	—	1	—	1	1
Total number of records	4	12	23	8	22	25	47	—
Species richness	3	6	10	3	8	7	12	—
Number of collections plated	10	10	10	10	20	20	40	—
Number of plates positive for protosteloid amoebae	3	4	5	3	6	9	15	—
% of plates positive for protosteloid amoebae	30	40	50	30	30	45	37.5	—
Abundance-based coverage	6	16	14	4	14	7	—	—
Incidence-based coverage	3	6	10	3	8	7	—	—
Sampling effort (%)	50	38	71	75	57	100	—	—

*TI = total identifications; NCP = number of collection points where the species was isolated.

Genus *Cavostelium* L.S. Olive 1965

***Cavostelium apophysatum* Olive 1965**

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 1; 16.6416°N, 120.3080°E; C.E. Ocenar-Bautista leg.; ground litter; 1 record, S1G2 • ibid.; point 2; 16.6479°N, 120.3108°E; N.H.A Dagamac leg.; aerial litter, 1 record, S2A3 • Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E.O. Bautista leg.; aerial litter; 2 records, S3A2, 2 records, S3A3; ground litter, 1 record, S3G4 * ibid.; point 4; 16.6533°N, 120.3180°E; N.H.A. Dagamac leg.; aerial litter; 3 records, S4A1.

Identification. This non-deciduous species has a single spherical spore that is supported by a short and wide stalk with a distinguishable cup-like apophysis that may also be indiscernible to the thickness of the stalk in some cases. The spore is less refractile compared to other protosteloid amoebae species due to its rough texture.

Genus *Echinostelium* de Bary 1873

***Echinostelium bisporum* (Olive & Stoian.) Whitney & Olive 1982**

New record. PHILIPPINES – La Union Province • San Fernando City, Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 1 record, S3A5.

Identification. One of the smallest known species of myxomycetes, this species is first described as a protosteloid species and is commonly cultured with them as they share the same habitat conditions. This species contains two spores per sporangium, which is arranged linearly, continuous to its short and stiff stalk.

Genus *Luapeleamoeba* Shadwick & Spiegel 2016

***Luapeleamoeba arachispora* (Olive & Stoian.) Tice & Brown 2016**
(= *Protostelium arachisporum* Olive & Stoian.)

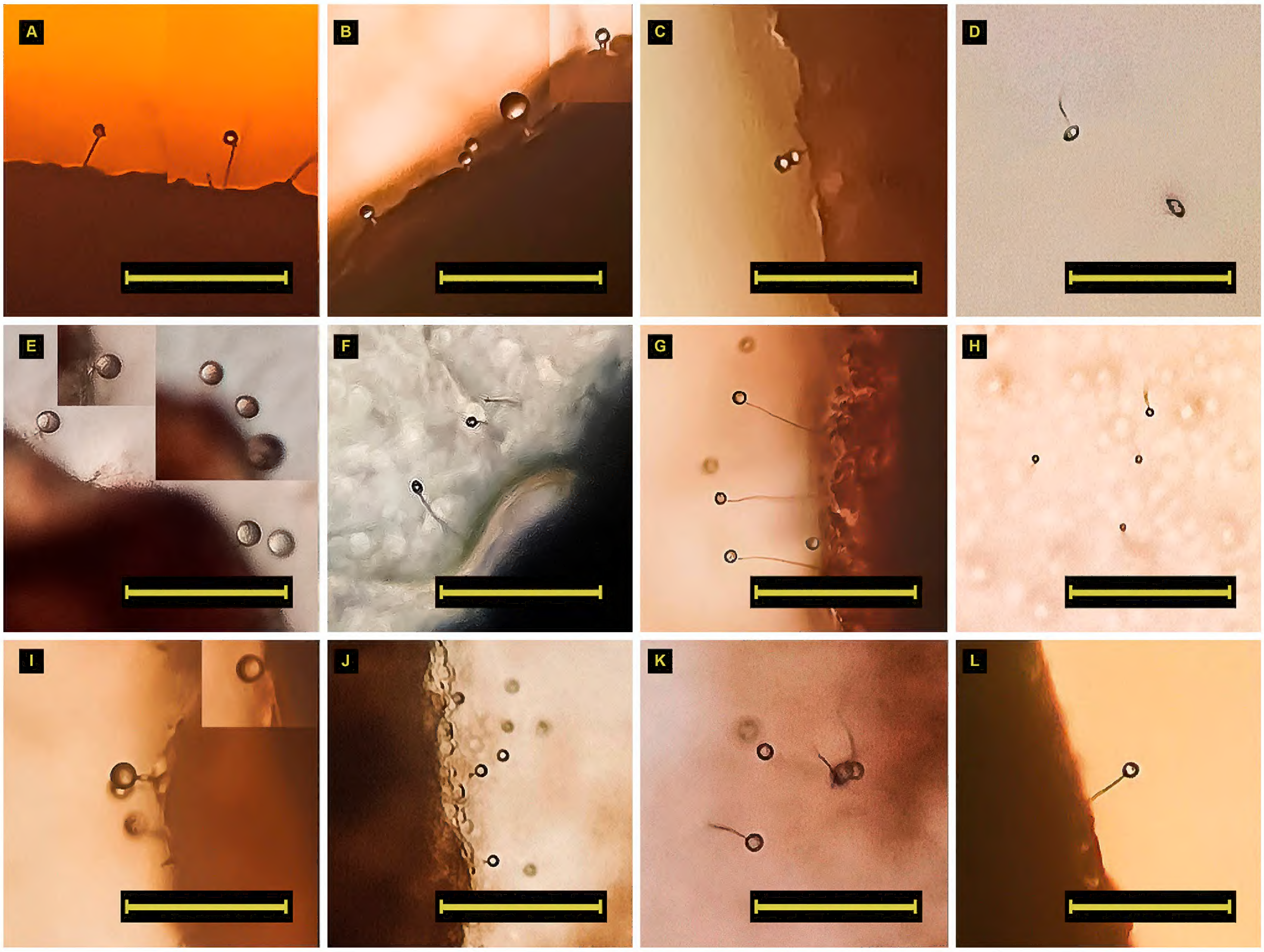

Figure 3. Protosteloid amoebae species recovered from San Fernando City, La Union, Philippines, viewed at 100× magnification (scale bars = 100 µm). **A.** *Acanthamoeba pyriformis.* **B.** *Cavostelium apophysatum.* **C.** *Echinostelium bisporum.* **D.** *Luapeleamoeba arachispora* **E.** *Microglomus paxillus.* **F.** *Nematostelium ovatum.* **G.** *Protostelium mycophagum.* **H.** *P. nocturnum.* **I.** *Schizoplasmodiopsis amoeboidea* **J.** *S. pseudoendospora.* **K.** *S. vulgaris.* **L.** *Tychosporium acutostipes.*

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 1 record, S3A5.

Identification. This species can be readily identified with its elongated peanut-shaped spore which is supported by a narrow stalk that slightly tapers towards its apex. While the size of this species has considerable variations, the recovered *L. arachispora* falls within the smaller bracket.

Genus *Microglomus* Olive & Stoian. 1977

Microglomus paxillus Olive & Stoian.1977

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Dalumpinas Oeste, point 2; 16.6479°N, 120.3108°E; N.H.A Dagamac leg.; aerial litter, 1 record, S2A1 • ibid.; point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 1 record, S3A2, 1 record, S3A5.

Identification. This multiple (2–4) spore-bearing species has a flattened spherical sporangium caused by the compression of the spores inside. The sporangium is supported by a short stalk. Individual spores are perceived following further examination through the sporangial sheath at a higher magnification.

Genus *Nematostelium* Olive & Stoian. 1970

Nematostelium ovatum (Olive & Stoian.) Olive & Stoian. 1970

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 1; 16.6416°N, 120.3080°E; C.E. Ocenar-Bautista leg.; ground litter, 1 record, S1G2, 1 record, S1G3 • ibid.; point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; ground litter, 1 record, S3G4.

Identification. This species contains a distinguishable ovoid to nearly ellipsoid deciduous spore which is supported by a long, robust stalk that appears refractile along its entire length.

Genus *Protostelium* Olive & Stoian. 1960

Protostelium mycophagum Olive & Stoian. 1960 species complex

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 1; 16.6416°N, 120.3080°E; C.E. Ocenar-Bautista leg.; ground litter, 1 record, S1G3 • ibid.; point 2; 16.6479°N, 120.3108°E; N.H.A. Dagamac leg.; ground litter, 2 records, S2G3 • Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 3 records, S3A2, 2 records, S3A5; ground litter, 1 record, S3G5 • ibid.; point 4; 16.6533°N, 120.3180°E; N.H.A. Dagamac leg.; ground litter, 1 record, S4G2.

Identification. This species complex is known for its variable morphology reflected in its spore size and deciduousness that is being held by a long stalk that appears to be flexuous at maturity. Nevertheless, the recovered *P. mycophagum* exhibits a morphotype similar to the type strain, distinguishing it from the *P. mycophagum rodmani* subclade which has a conspicuous thick basal section and a thin basal portion (Shadwick et al. 2018).

Protostelium nocturnum Spiegel 1984

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; ground litter, 2 records, S3G4 • ibid.; point 4; 16.6533°N, 120.3180°E; N.H.A. Dagamac leg.; ground litter, 4 records, S4G1.

Identification. The stalk of this species disappears following the discharge of its spherical spore, thus classifying the species as ballistosporous. This species is among the smallest of protosteloid amoebae.

Genus *Schizoplasmodiopsis* Olive 1967

Schizoplasmodiopsis amoeboidea (Olive & Stoian.) Spiegel 1994

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 2 records, S3A2.

Identification. This short-stalked species is characterized by a sudden taper at the apex of its stalk accompanied by a disproportionately large spherical spore—one of the largest among any protosteloid amoebae.

Schizoplasmodiopsis pseudoendospora Olive, Martin & Stoian. 1967

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 2; 16.6479°N, 120.3108°E; N.H.A. Dagamac leg.; ground litter, 5 records, S2G3.

Identification. One of the smallest known species of protosteloid amoebae, the stalk of this species is very short, which does not narrow at the point of attachment to its small spherical spore.

Schizoplasmodiopsis vulgaris Olive & Stoian. 1976

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 2; 16.6479°N, 120.3108°E; N.H.A. Dagamac leg.; ground litter, 1 record, S2G3 • Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 1 record, S3A2; ground litter, 1 record, S3G4.

Identification. The stalk of this species is characterized by its thickness along its entire length which slightly tapers towards its spherical, coarse-looking spore.

Genus *Tychosporium* Spiegel 1995

Tychosporium acutostipes Spiegel, Moore & Feldman 1995

New records. PHILIPPINES – La Union Province • San Fernando City, Barangay Lingsat, point 1; 16.6416°N, 120.3080°E; C.E. Ocenar-Bautista leg.; ground litter, 1 record, S1G1 • Barangay Dalumpinas Oeste, point 3; 16.6511°N, 120.3158°E; C.E. Ocenar-Bautista leg.; aerial litter, 1 record, S3A5.

Identification. The long stalks of this species appear to be stiff and thick which only taper sharply at its apical region articulating with a spherical spore.

DISCUSSION

Our study reveals a relatively moderate level of species richness (12 spp.) in comparison with past sampling efforts carried out elsewhere in the world, whereby this number of species accounts for approximately 32% of the described known protosteloid amoebae species (Shadwick et al. 2009a, 2016). Similar levels of species richness that have been documented in the tropics include: 16 species recovered from the tropical wet forests of Costa Rica (Stephenson and Moore 1998; Moore and Stephenson 2003), 13 species from the tropical forests of Puerto Rico (Stephenson et al. 1999; Moore and Spiegel 2000b), 12 species from forests, woodland, and deserts in Australia (Powers and Stephenson 2006), and 15 species from forest areas in Tanzania and Malawi (Ndiritu et al. 2009). This moderate degree of species richness recovered by us can be linked with the limited heterogeneity of plant communities within the study area which features a dominance of coconut and mangrove trees (Balaoro-Banzuela et al. 2023).

While our study may be the first of its kind to be conducted in the paleotropics, a number of generalizations can be made owing to the advent of ecological studies focusing on this group of species in other parts of the world. *Protostelium mycophagum* is universally acknowledged as a cosmopolitan species due to its widespread distribution, being reported only as either abundant or common. *Cavostelium apophysatum* is also a widely present species but has a preference towards tropical conditions as shown in studies classifying the species as either abundant or common (Stephenson and Moore 1998; Stephenson et al. 1999; Moore and Spiegel 2000b; Moore and Stephenson 2003; Powers and Stephenson 2006; De Haan et al. 2014), although some surveys also designate the species as occasional or rare (Ndiritu et al. 2009). Additionally, *A. pyriformis* was also recovered in both temperate and tropical regions but is never abundant nor common in these areas. With respect to their microhabitats, *Schizoplasmodiopsis pseudoendospora* is predominantly isolated in ground-litter substrates, which is consistent with established trends in several studies (Powers and Stephenson 2006; Ndiritu et al. 2009; Aguilar et al. 2011). However, there are also some notable microhabitat preference differences that can be drawn from the occurrences of *P. nocturnum* which shows a preference for ground litter, as well as *C. apophysatum*, which otherwise prefers aerial litter. This warrants further investigation to elucidate whether this is a sampling error or a true pattern.

Nevertheless, the results of this pioneering study provide baseline information to disentangle patterns on the occurrence and abundance of protosteloid amoebae in the paleotropics for each microhabitat. This is the first effort to investigate the occurrence of protosteloid amoebae in the country as well as in Southeast Asia, which may foster future studies of ecological and environmental implications.

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ADDITIONAL INFORMATION

Conflict of interest
The authors declare that no competing interests exist.

Ethical statement
No ethical statement is reported.

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Author contributions
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Data availability
All data that support the findings of this study are available in the main text.

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